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NOREPINEPHRINE INVOLVEMENT IN PENILE DETUMESCENCE

W. DIEDERICHS,* C. G. STIEF, T. F. LUE AND E. A. TANAGHO

From the Department of Urology, University of California School of Medicine, San Francisco, California

ABSTRACT

Adrenergic neurotransmission was studied in dogs. Blood samples for catecholamine assay were collected from the flaccid penis, the erect penis after cavernous nerve stimulation and during detumescence induced by sympathetic trunk stimulation. Epinephrine concentration was not significantly different in these three stages. However, norepinephrine concentration increased significantly ($p < 0.005$) during induced detumescence from 505±311[pg./ml.] to 747±489 [pg./ml.].

Intracavernous application of norepinephrine abolished cavernous nerve induced erection in a dose dependent manner. Sympathetic trunk stimulation reduced or abolished erection induced by acetylcholine or by vasoactive intestinal polypeptide. We conclude that norepinephrine is an important neurotransmitter in the control of penile detumescence. (*J. Urol., 143: 1264–1266, 1990*)

Various protocols of neurostimulation[1] and intracavernous (i.c.) injection of various neurotransmitters[2] can elicit erection and detumescence. For example, acetylcholine (Ach), norepinephrine and vasoactive intestinal polypeptide (VIP) have been extensively used in studies on erection.[2,3,4] In dogs, erection can be induced by i.c. application of Ach[5], VIP[3], and also by pelvic[6] or cavernous nerve stimulation.[7] Penile detumescence has been induced by sympathetic trunk stimulation[1,8] and/or i.c. norepinephrine injection.[2] These results suggest that neurostimulation is effective on penile tissue probably via the release of one of the neurotransmitters mentioned above.

The present study was designed to clarify, in vivo, adrenergic effects on erection. We measured epinephrine (E) and norepinephrine (NE) levels in penile blood during erection and detumescence phases induced by neurostimulation. We also compared effects of neurostimulation to the action of (i.c.) injected neurotransmitters.

MATERIALS AND METHOD

Experiments were performed on sixteen adult male mongrel dogs (19.2 to 43.3 kg.). The animals were premedicated with acepromazine (0.1 mg./kg.) and ketamine (five mg./kg.) subcutaneously and anesthetized with pentobarbital (i.v., 130 mg. as a bolus; additional doses as required). Fluid was maintained by infusion of saline at 100 ml./hr. The dogs were placed in the supine position on a water heated pad at 39C, and the lower abdomen was exposed by a midline incision. The lumbar sympathetic trunks were separated from the aorta and fitted with bipolar cuff electrodes (Avery Laboratories). The right and left cavernous nerves were identified close to the prostate, and cuff electrodes were placed around them. The right internal pudendal artery was fitted with an ultrasonic flow probe (Transonics Systems, Inc., Ithaca, NY). The midshaft of the penis was dissected free from the skin and the subcutaneous layers.

Each corpus cavernosum was punctured with two 19 gauge scalp vein needles. One needle was used to collect blood samples from the penis and the other for recording the pressure. Systemic blood pressure was recorded through a 16 gauge angiocath needle placed in the right femoral artery. One scalp vein needle from each corpus and the angiocath needle were connected to Statham pressure transducers. A Grass polygraph (Model 7) was used for recording the pressures.

A transmitter with an external antenna (Avery Laboratories) was used for neurostimulation (parameters: 20 Hz; 200 microseconds per pulse; voltage range 1.2 to 3 V; each stimulation for 60 seconds).

Systemic blood samples (one cc) were collected from the cephalic vein ten minutes after application of the premedication (sample 1), from the femoral artery after the surgical set-up (sample 2) and immediately after sympathetic trunk stimulation (STS; sample 3). Penile samples from the corpora cavernosa were taken before (sample 4) and after sixty seconds of cavernous nerve stimulation (CNS; sample 5) as well as thirty to forty seconds after initiation of STS (sample 6). The blood was withdrawn into syringes containing EGTA and glutathion, stored in ice, centrifuged (4C, 1000 g for 20 minutes), and the plasma separated and stored at 120C. To measure the catecholamine plasma levels a radioenzymatic assay[9] was used. The assay can detect one pg. (20 pg./ml. of plasma) of NE or E.

The following neurotransmitters were used for (i.c.) injection after dilution with saline to 0.3 to 0.5 ml.:

Acetylcholine: Cooper Vision, Pharmaceutical, Inc. (Dose: 10–50 μg.)

Norepinephrine: Wintrop-Breon Lab, (Dose: 10–50 μg.)

VIP (porcine synthetic): Sigma (Dose: 10–20 μg.)

Data are expressed as mean or as mean ± standard deviation unless otherwise stated. The concentrations from the systemic blood (sample 1–3 = group 1) as well as from penile blood (sample 4–6 = group 2) were compared with a Wilcoxon signed rank test. Values were considered significantly different when $p < 0.05$.

RESULTS

The intracavernous pressure increased almost ten-fold within one minute after the start of cavernous nerve stimulation. Recovery to the baseline pressure after the cessation of stimulation typically required five to six minutes. However, when the sympathetic trunks were stimulated 10 to 15 seconds after the cessation of nerve stimulation, the recovery required only on the order of 30 seconds. Rate of recovery was hence about ten times faster with as compared to without sympathetic trunk stimulation.

Surgical stress significantly increased systemic blood concentrations of E and NE, but neurostimulation did not further increase catecholamine levels. In penile blood, however, STS significantly increased NE when compared with the concentration found after CNS (see table 1).

Figure 1 A shows the increase (in two distinct steps) in the intracavernous pressure following the i.c. injection of acetylcholine (Ach). Recovery of the baseline pressure generally required on the order of one to two minutes, as shown.

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* Requests for reprints: Urologische Klinik, Marienhospital Herne, Universitätsklinik, Ruhr Universität Bochum, Widumerstr. 8, 4690 Herne, West Germany.

TABLE 1. *Epinephrine (E) and norepinephrine (NE) values*

Sample	E [pg./ml.] Mean	SD	NE Mean	SD
1	224*	49	298†	158
2	695*	337	803†	416
3	982	574	804	487
4	426	251	398	264
5	513	270	505§	311
6	556	304	747§	489

§ †,* Pairs indicated by these symbols consist of two significantly different means ($p < 0.05$).

Sample 1: from cephalic vein — ten minutes after premedication
2: from femoral artery — after surgical set up
3: from femoral artery — after STS
4: from cavernous body — (no stimulation)
5: from cavernous body — after CNS
6: from cavernous body — after STS

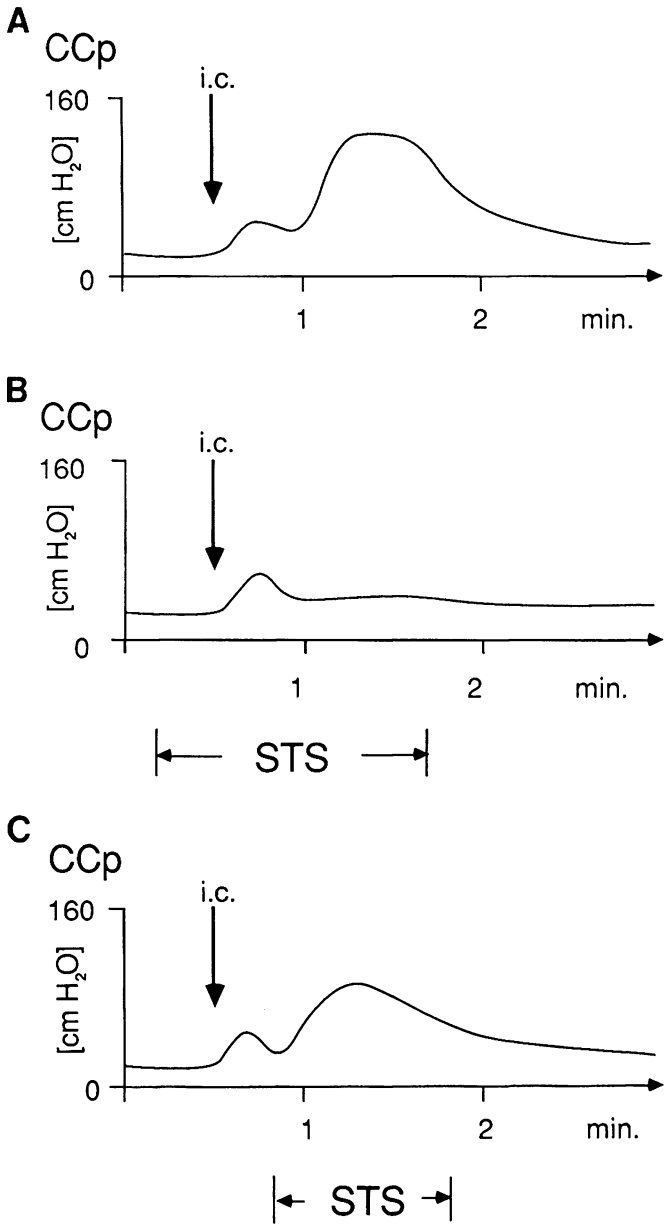

FIG. 1. Effect of 10 μg. Ach (arrow: intracavernous injection) and sympathetic trunks stimulation (STS) on intracavernous pressure (CCp).

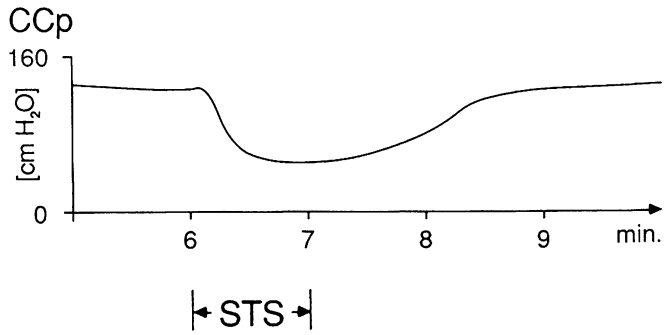

FIG. 2. VIP induced erection and sympathetic trunks stimulation (STS) on intracavernous pressure (CCp).

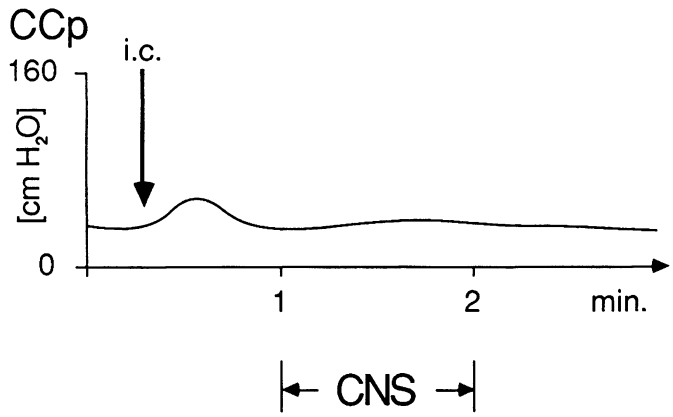

FIG. 3. Effect of intracavernous injection of norepinephrine (arrow) and cavernous nerve stimulation (CNS) on intracavernous pressure (CCp).

If the sympathetic trunks were stimulated before, during, and after the injection of 10 μg. Ach, however, the Ach had no effect other than the small changes shown in figure 1B. STS partially negated the effect of injection of a much larger dose of Ach (50 μg.).

If the sympathetic trunks were stimulated after (instead of before, during, and after) 10 μg. Ach injection, the intracavernous pressure increase was attenuated as compared with no STS (figure 1C). STS had no effect on the i.c. pressure after injection of 50 μg. Ach.

VIP (five dogs). One to three minutes after VIP injection, the i.c. pressure increased from about 20 cm. H_2O to 90 cm. H_2O. The concomitant erections lasted between four and twenty minutes. STS occuring three, six, 10 and 15 minutes after VIP injection caused the i.c. pressure to decrease (figure 2), with the pressure drop less after three minutes than after six, 10, and 15 minutes. Recovery of the elevated pressures and the erect penis required longer times after STS was repeated several times. For example, the time required to recover the maximum i.c. pressure increased from 130 seconds after two episodes of STS to 270 seconds after four episodes.

Norepinephrine (six dogs). In two dogs a slight i.c. pressure increase (10 cm. H_2O to 15 cm. H_2O) was seen after i.c. injection of either 10 μg. or 50 μg. of NE. Forty seconds after NE injection, cavernous nerve stimulation resulted in no i.c. pressure rise in two dogs and a slight response (to a peak pressure of 36 cm. H_2O) in the other four dogs (figure 3).

DISCUSSION

Catecholamine serum levels in conscious and anesthetized dogs were studied by Bridle et al.[10], and the concentrations of epinephrine and norepinephrine that we found in the periphery are about 40% higher than their findings. Our elevated E and NE levels are from blood samples taken when the abdominal

cavity was open, and the nerves and penis were exposed. This surgical stress might be the source of the relatively elevated levels we observed. However, a side dependence from our E and NE levels can not be excluded because the first samples were collected from the cephalic vein and the second samples from the femoral artery.

Ultrastructural examination of the erectile tissue showed that smaller granular vesicles, which are believed to contain norepinephrine, lay close to the smooth muscle cells of the penis.[11] After the release of NE, its effects are attenuated by reuptake into the pre-synaptic nerve terminals, metabolic transformation, and dilution by diffusion out of the junctional cleft.[12] The transmitter level assayed in the cavernous blood reflects all these release and elimination phenomena. We do not know whether the cavernous blood levels of transmitters correlate with the amount of synaptic release of transmitters. However, we do believe that the elevated plasma levels of norepinephrine during STS is a reflection of the norepinephrine released by the neural vesicles.

Blood samples from the penis were collected before and during various neurostimulations. Catecholamine levels in blood from a flaccid penis were much lower than from the femoral artery. This might be due to low arterial inflow into the cavernous spaces during the flaccid state and a lower rate of synaptic release. When tumescence was induced by CNS there was a large inflow of blood containing catecholamines at higher concentrations than in flaccid penile blood. Nevertheless, we found an increase only of NE and not of E during the tumescence phase. NE levels continued to increase during detumescence, when arterial inflow decreases.[7]

We collected blood samples while hemodynamic changes were occurring in the penis (owing to neurostimulation) in order to correlate catecholamine levels to the different phases of erection. Using a lower voltage for neurostimulation, one might expect to observe an increase of the norepinephrine level but not a reduction in the intracavernous pressure,[1] the latter of which is related to a tension of the smooth muscles.[2] In a previous study[1] we suggested that the cavernous nerve is composed of parasympathetic and sympathetic fibers. This composition might explain elevated norepinephrine levels during cavernous nerve stimulation. These norepinephrine levels seem too low to abolish erection. Furthermore, our results show that the sympathetic inhibition of erection occurs at smooth muscle level. Previously Langley and Anderson[8] observed a strong contraction of the muscles by sympathetic trunk stimulation when they cut open the penile bodies of cats and rabbits. In addition they described a contraction of penile arteries and a decrease in size of the penis. Later Semans and Langworthy[13] noticed the subsidence of erections within a few seconds by STS. According to these two landmark articles of sympathetic trunks effects Carati et al.[14] showed a reduction of STS effects by the alpha-adrenergic antagonist phentolamine as well as by the $alpha_1$-adrenergic antagonist prazosin. We suggest that these findings form a link between the norepinephrine release and the demonstration of alpha adrenergic receptors in penile tissue.[15]

In our experiments exogenous NE decreased (10 μg. i.c.) or abolished (50 μg. i.c.) cavernous nerve induced erection. This effect on the intracavernous pressure is similar to the effect of sympathetic trunk stimulation.[1] The slight i.c. pressure increase observed after NE i.c. injection is similar to a small i.c. pressure rise by STS as a result of a strong smooth muscle contraction in the cavernous bodies.[1] The relationship of dosage to the degree of smooth muscle contraction has been shown in a muscle bath.[16] It is generally believed that several transmitters are involved in the autonomic transmission[17] and NE is probably not the only transmitter in the smooth muscle contraction process.

The relaxation effect of acetylcholine on penile smooth muscles is controversial,[5,18] especially in vitro.[16,19] Hedlund and Andersson[16] mentioned that pelvic nerve activity leading to a release of Ach may contribute to relaxation of tissue by counteracting the postjunctional effects of NE. This effect might also be important on the reduction of VIP induced erection by STS. The intracavernous pressure increase during VIP was only of about 65% compared to the maximum pressure by Ach or CNS.

We conclude that the inhibition of penile erection during STS is due to a release of norepinephrine in the penile tissue. STS also inhibits tumescence induced by intracavernous injection of VIP or acetylcholine through the same mechanism. Whether acetylcholine and/or VIP are involved in the tumescence process is still under investigation, but our results seem to indicate that norepinephrine is definitely involved in the detumescence process.

REFERENCES

1. Diederichs, W., Stief, C. G., Benard, F., Bosch, R., Lue, T. F. and Tanagho, E. A.: Sympathetic effect on erection in dogs and monkeys. J. Urol., **139**:253A, 1988.
2. Lue, T. F. and Tanagho, E. A.: Physiology of erection and pharmacological management of impotence. J. Urol., **137**:829, 1987.
3. Juenemann, K. P., Lue, T. F., Luo, J., Jadallah, S. A., Nunes, L. L. and Tanagho, E. A.: The role of vasoactive intestinal polypeptide as a neurotransmitter in canine penile erection: a combined in vivo and immunohistochemical study. J. Urol., **138**:871, 1987.
4. Lincoln, J., Crowe, R., Blaklay, P. F., Pryor, J. P., Lumley, J. S. P. and Burnstock, G.: Changes in the vipergic, cholinergic and adrenergic innervation of human penile tissue in diabetic and non-diabetic impotent males. J. Urol., **137**:1053, 1987.
5. Valiji, K. and Bookstein, J. J.: The veno-occlusive mechanism of the canine corpus cavernosum: angiographic and pharmacologic studies. J. Urol., **138**:1467, 1987.
6. Holmquist, B. and Olin, T.: Electrostimulation in male dogs at pelvic nerve stimulation. Scan. J. Urol. Nephrol., **2**:115, 1968.
7. Lue, T. F., Takamura, T., Umraiya, M., Schmidt, R. A. and Tanagho, E. A.: Hemodynamics of canine corpora cavernosa during erection. Urol., **24**:347, 1984.
8. Langley, J. N. and Anderson, H. K.: The innervation of the pelvic and adjoining viscera. Part III. The external generative organs. J. Physiol. **19**:85, 1895.
9. Peuler, J. D. and Johnson G. A.: Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine. Life Sci., **21**:625, 1977.
10. Bridle, P. A., Brosnihan, K. B., Speth, R. C., Goormastic, M. and Ferrario, C. M.: Basal levels of plasma epinephrine and norepinephrine in the dog. Supp V, Hypert., **5**:128, 1983.
11. Benson, G. S., McConnell, J. and Lipshultz, L. I.: Neuromorphology and Neuropharmacology of the human penis. J. Clin. Invest., **65**:506, 1980.
12. Mayer, S. E.: Neurohumoral transmission and the autonomic nervous system. In: The Pharmacological Basis of Therapeutics. Edited by A. Goodman Gilman, A. Goodman and L. S. Gilman. New York: Macmillan Publ. Inc., vol. 7, chap. 4, pp. 56–90, 1985.
13. Semans, J. H. and Langworthy, O. R.: Observations on the neurophysiology of sexual functions in the male cat. J. Urol., **40**:836, 1938.
14. Carati, C. J., Creed, K. E. and Keogh, E. J.: Autonomic control of penile erection in the dog. J. Physiol., **384**:525, 1987.
15. Levin, R. M. and Wein, A. J.: Adrenergic alpha receptors outnumber beta receptors in the human penile corpus cavernosum. Invest. Urol., **18**:225, 1980.
16. Hedlund, H. and Andersson, K. E.: Comparison of the responses to drugs acting on adrenoreceptors and muscarinic recepters in human isolated corpus cavernosum and cavernous artery. J. Auton. Pharmac., **5**:81, 1985.
17. Burnstock, G.: The changing face of autonomic neurotransmission. Acta Physiol. Scand., **126**:67, 1986.
18. Dorr, L. D. and Brody, M. J.: Hemodynamic mechanisms of erection in the canine penis. Am J. Physiol., **213**:1526, 1967.
19. Adaikain, P.G., Karim, S. M. M., Kottegoda, S. R. and Ratman, S. S.: Cholinoreceptors in the corpus cavernosum muscle of the human penis. J. Auton. Pharmac., **3**:107, 1983.